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Abstract

The ecology of peatland testate amoebae is well studied along broad gradient from very wet (pool) to dry (hummock) micro-sites where testate amoeba are often found to respond primarily to the depth to water table (DWT). Much less is known on their responses to finer-scale gradients and nothing is known of their possible response to phenolic compounds, which play a key role in carbon storage in peatlands. We studied the vertical (0-3 cm; 3-6 cm; 6-9 cm sampling depths) micro-distribution patterns of testate amoebae in the same microhabitat (*Sphagnum fallax* lawn) along a narrow ecological gradient between a poor fen with an almost flat and homogeneous *Sphagnum* carpet (fen) and a "young bog" (bog) with more marked micro-topography and mosaic of poor-fen and bog vegetation. We analysed the relationships between the testate amoeba data and three sets of variables (1) "chemical" (pH, Eh potential & conductivity), (2) "physical" (water temperature, altitude i.e. *Sphagnum* mat microtopography & DWT) and (3) phenolic compounds in/from *Sphagnum* (water-soluble and primarily bound phenolics) as well as the habitat (fen/bog) and the sampling depth. Testate amoeba Shannon H' diversity, equitability J of communities, and total density peaked in lower parts of *Sphagnum*, but the patterns differed between the fen and bog micro-sites. Redundancy analyses (RDA) revealed that testate amoeba communities differed significantly in relation to Eh, conductivity, water temperature, altitude, water-soluble phenolics, habitat, and sampling depth, but not to DWT, pH, or primarily bound phenolics. The sensitivity of testate amoebae to weak environmental gradients makes them particularly good integrators of micro-environmental variations and has implications for their use in paleoecology and environmental monitoring. The correlation between testate amoeba communities and the concentration of water-soluble phenolic suggests direct (e.g. physiological) and/or indirect (e.g. through impact on prey organisms) effects on testate amoebae, which requires further research.

Introduction

Testate amoebae are abundant and diverse shelled protozoa living in a wide range of habitats ranging from soils, lakes, rivers, wetlands, and moss habitats [4, 13, 62]. Owing to ecological gradients and the preservation of their shells in peat and sediments, these protists are useful proxies in paleoenvironmental and ecological studies of peatland and lakes [6, 11, 43]. In *Sphagnum* bogs, testate amoeba community composition is generally strongly correlated to surface wetness conditions (mostly assessed by the water table depth – hereafter DWT) and water chemistry [3, 39, 48, 59].

While the relationship between testate amoebae and DWT, and a few other variables such as pH are well documented along broad ecological gradient (e.g. wet pools to dry hummocks, fen to bog) [26, 47], much less is known on their finer-scale responses to micro-environmental gradients. Some data suggests that testate amoebae may be highly sensitive even to subtle micro-environmental gradients. For example Mitchell et al. [40] studied the horizontal distribution patterns of testate amoeba communities in a 40x60cm almost flat mono-specific *Sphagnum* lawn and found spatial heterogeneity in the communities that was significantly correlated to altitude (microtopography) (despite a very short – ca. 6cm – elevation gradient). Assessing testate amoeba species-environment correlation along fine-scale environmental gradients is necessary to define the practical limits (i.e. the resolution) of their use as bioindicators in ecological and palaeoecological studies.

Another open question is the range of abiotic and biotic factors to which testate amoebae respond. Although many variables have been studied, DWT almost always emerges as the strongest variable despite the fact that testate amoebae are unlikely to be directly influenced by the position of the water table 10 or 30 cm below the level where they live [41]. Still some important potential factors have not yet been studied including peat and water chemistry beyond simple ions and elements. *Sphagnum* peatlands are indeed generally

characterized by gradients such as nutrients (nutrient-poor ombrotrophic bogs vs. rich fens), hydrology (wet hollow vs. dry hummocks) and acidity [14, 22, 23, 52].

Recently, phenolic compounds (secondary metabolites) produced by plants have been described to play an important role in the interactions of plants with their environment including microorganisms [24]. For example in humus spruce forests such compounds have been shown to cause the increase of several microbial communities (i.e. cellulose hydrolyser) and in the decrease of others (i.e. bacteria) [56, 57]. While the production of phenolic compounds by vascular plants is well documented, few studies have addressed phenols production by non-vascular cryptogams such as *Sphagnum.* The role of phenolics produced by vascular plants on the functioning of the bog ecosystem is established [18], as well as the phenolics content gradient between knoll forest-peat bogs and peat bogs [16]. Possible effects of phenolics produced by *Sphagnum* on microorganisms, including testate amoebae, are still unknown. *Sphagnum* contains weakly as well as primarily bound phenolics to the cell wall [61]. The unique morphology and anatomy of *Sphagnum,* allows water-soluble phenols to be easily released in the *Sphagnum* surrounding environment. Thus the patterns of phenol concentrations at the surface of *Sphagnum* peatlands may contribute to creating micro-patterned habitats and a range of ecological niches suitable for the establishment of diverse communities of organisms including testate amoebae [1, 12, 40].

The aims of this study are to explore (1) the species-environment relationships and (2) vertical micro-distribution patterns of testate amoebae along a short ecological gradient from a *Sphagnum*-dominated poor fen (for simplicity hereafter referred to here as “fen”) and a vegetation with mixed bog and poor fen plant elements and a more marked micro-topography (hereafter referred to as “bog”). Rather than sampling contrasted microhabitats or moss species, we sampled only within macroscopically homogenous and similar *Sphagnum fallax* carpets across the gradient. We assessed (1) how horizontal and vertical patterns of testate

amoebae community structure varied along the gradient, and (2) the relationships between the testate amoeba communities and DWT, water chemistry and phenolic compound content. We hypothesized (1) that the vertical patterns of community structure would be more marked in the structurally more complex mixed *Sphagnum* "bog" habitat than in the more uniform poor fen, despite the fact that the sampled habitats were macroscopically identical and, (2) that phenolic compounds would explain a similar fraction of the community data structure as other more commonly studied environmental factors (i.e. altitude, DWT, water chemistry).

Methods

Sampling and laboratory analyses

The study site was an undisturbed ombrotrophic *Sphagnum*-dominated mire [2] situated in the Jura Mountains (The Forbonnet peatland, France, 46°49'35''N, 6°10'20''E) at an altitude of 840 m above sea level (Supplementary Fig. 1). Cold winters (on average of -1.4°C) and mild summers (on average of 14.6°C) characterized the climate of the site. The annual mean temperature measured at the site over a one year period from November 5th 2008 to November 30th 2009 was 6.5°C and the annual precipitations were 1200 mm.

Samples of *Sphagnum fallax* were collected from two adjacent areas (ca. 10 m x 12 m) selected in relation to their micro-topography, vegetation and assessment of sources and decay of organic matter [15]. The first sampling area (coded "fen") is a transitional *Sphagnum*-dominated poor fen area, relatively flat and homogeneous, characterized by a moss cover dominated by *Sphagnum fallax* and by the lack of *S. magellanicum*. Vascular plants as *Eriophorum vaginatum*, *Vaccinum oxycoccus* and *Andromeda polifolia* were recorded in very low abundance. *Scheuchzeria palustris* and *Carex limosa* occurred outside of the studied plots. The second sampling area (coded "bog") is an open bog area with mixed vegetation,

directly adjacent to the fen area. Patterns of hummocks with *S. magellanicum, V. oxycoccos, E. vaginatum, C. rostrata* and *Calluna vulgaris,* and hollows with lawns of *S. fallax* and *A. polifolia* characterized the sampling area. The terms "fen" and "bog" are used here for simplicity and to denote the existence of a trophic gradient inferred from the vegetation. However the "bog" sub-site represents a mosaic of poor-fen (lawns, hollows) and bog (hummock) vegetation.

In each of the two sampling areas, six plots were selected in representative surfaces. Among the 12 sampling plots, the maximal distance between the two most distant plots was ca. 30 m. On June 26th 2008, samples of *S. fallax* were collected in each plot for the study of testate amoeba communities and phenolic compounds around 10 permanent markers in each plot. The goals of this sampling design were (1) to allow for multiple sampling at the site over time (this study representing the T0 of a warming experiment), and (2) to obtain a composite sample from each plot and avoid any bias due to spatial heterogeneity [40]. Moreover in each plots, the Eh potential, the pH, the conductivity (K), the water temperature (W-Temp), the depth to the water table (DWT; measured in a piezometer in the centre of each plot), and the average altitude (microtopography, Alt) of the sampled plot were measured. To assess the effect of microtopography on spatial distribution patterns, the average altitude (in millimeters) of the 10 permanent markers was recorded in each sampling plots using an arbitrary reference [40]. The values of pH and conductivity were standardized to 20°C. The conductivity caused by hydrogen ions was subtracted according to Sjörs [55]. Corrected conductivity (Kcorr) was then used as a proxy for total mineral richness of the water.

Primarily bound (hereafter "bound") and water-soluble phenolic (hereafter "free") compounds were extracted and quantified from lyophilized mosses. The green section (0-6 cm; 0 being defined as the top of the capitulum) was used for these analyses, excluding the lower part where the mosses start to decay. Two methods were used to extract phenolic

compounds from *Sphagnum*. For free phenolics, 0.05g dry weight (DW) of *Sphagnum* was ground in a mortar, mixed with 10 mL distilled water, bubbled with nitrogen and agitated on a reciprocal shaker (15 rpm) for 3 hours and filtered. For bound phenolic compounds, 0.05g DW of *Sphagnum* was ground in a mortar, mixed with 25 mL ethanol / distilled water (80/20 v/v) and warmed under reflux at 120°C for 30 minutes. This extract was filtered and evaporated by using a rotary evaporator. Finally, the dry extract was dissolved in 25 mL of boiling distilled water (adapted from Gallet and Lebreton [19]). The free and bound total phenolic contents were quantified with the Folin-Ciocalteau reagent and were expressed in mg equivalent gallic acid (A_{760}).

For testate amoeba analysis, the *Sphagnum fallax* samples were cut in three levels (sampling depth): 0-3 cm (upper), 3-6 cm (intermediate), and 6-9 cm (lower). The samples were fixed with 20 mL glutaraldehyde (2% final concentration) and stored at 4°C in the dark. Testate amoebae were extracted from mosses using the following extraction method [45]: each sample was shaken for 1 min on a vortex and then pressed to extract microorganisms (first solution). The mosses were then soaked again with 20 mL of glutaraldehyde (2%), shaken a second time on a vortex and pressed to extract *Sphagnum* leachate. The leachate was left to settle for 12h, after which the supernatant was added to *Sphagnum* and the bottom to the first solution. The process was repeated six times, and all fractions were combined to obtain a final composite sample of 40 mL. The remaining fraction was dried at 80°C for 48h and weighted to express testate amoeba density by gram dry weight (DW) of *Sphagnum*. The testate amoebae were identified and counted to a total of 150 at x200 and x400 magnification by inverted microscopy (OLYMPUS IX71) following Uthermöhl's method [60]. Testate amoebae were identified to the species level whenever possible. Only living amoebae (active only, encysted individuals were not included) were counted.

150 Numerical analyses

151 Total density, species richness (S), diversity index (the Shannon index H') and equitability
152 index (J) were calculated. Because the distributions of these data were not normal, non-
153 parametric Friedman tests were performed.

154 In all analyses, species that occurred in less than 2% of maximum density were
155 removed from the data set to reduce the influence of rare taxa on multivariate analyses [32].
156 We analyzed differences among sampling depths and between the fen and bog zones (nominal
157 variables) for the dominant testate amoeba species using a MANOVA test.

158 For all multivariate analyses, a Hellinger transformation was applied to stabilize the
159 variance and reduce the influence of the dominant taxa [33]. A Non-metric multidimensional
160 scaling (NMDS) was used to assess patterns of variation in testate amoeba community
161 structure along the different segments of *Sphagnum* (upper, intermediate and lower segments)
162 and between the fen and bog zones. As this analysis revealed clear differences among
163 sampling depths and between "fen" and "bog" zones ($P < 0.001$), we further explored the
164 species-environment correlations for the different sampling depths and in the two zones
165 separately as well as conducting global analyses.

166 Multiple factor analysis (MFA) was used to assess the general structure of the data and
167 to determine the relationships among the three Hellinger-transformed testate amoeba data sets
168 and the three environmental variables data sets (chemical, physical and phenolics) [17]. MFA
169 was performed in two steps. Firstly, a PCA was performed on each subset, which was then
170 normalized by dividing all its elements by the first eigenvalue obtained from its PCA.
171 Secondly, the normalized subsets were assembled to form a unique matrix and a second PCA
172 was performed on this matrix. RV-coefficient (ranging from 0 to 1) was used to measure the
173 similarity between the geometrical representations derived from each groups of variables [51].
174 RV-coefficients are then tested by permutations [29]. Euclidean distances of global PCA were

used in MFA to perform cluster analysis according to the Ward method, and the resulting dendrogram was projected in the MFA ordination space. This analysis revealed the main differences in the structure of the data described by all biotic and abiotic subsets of variables.

We assessed the relationships among the testate amoeba communities in the upper, intermediate and lower sampling depth and the three sets of environmental variables (1) "chemical" (pH, Eh potential & conductivity), (2) "physical": (water temperature, altitude & DWT) and (3) phenolic compounds (bound and free). The ordination patterns of testate amoeba communities and their causal relationships to environmental data-sets were assessed using redundancy analysis (RDA) [58]. The proportion of variance explained by environmental variables was quantified using variance partitioning. Adjusted R^2 were used in all RDA to estimate the proportion of explained variance [49]. The analysis was repeated with the sampling area and sampling depth data-sets transformed to presence/absence in order to reveal only testate amoeba communities differences.

All multivariate analyses were performed with the software R [50] using vegan [47] and FactoMineR [28] packages.

Results

Environmental variables

The range of values for the eight measured environmental variables, minimum, maximum and averages for the "fen" and "bog" areas are given in Table 1. The Eh potential and water temperature were significantly higher in the "fen" area while altitude and free phenols were significantly higher in the "bog" area ($P < 0.05$). Water pH, conductivity, DWT and the concentration of slightly bound phenolic compounds did not differ significantly between the two areas. All environmental variables, except Kcorr, pH, DWT and altitude, were

199 significantly correlated to free phenolics (Table 2) while no environmental variables were
200 significantly correlated to primarily bound phenolics.

201

202 Testate amoeba density and diversity

203 The total density of testate amoebae increased significantly with depth in the "bog" area from
204 3.2 x 10^4 ind.g^{-1} DW in the upper segments to respectively 7.45 x 10^4 and 10 x 10^4 ind.g^{-1}
205 DW in the intermediate and lower segments ($P < 0.05$). By contrast there was no significant
206 difference with depth in the "fen" area (average density over the three depths: 4.34 x 10^4
207 ind.g^{-1} DW).

208 A total of 28 testate amoeba taxa were identified in the 36 samples analyzed. In the
209 "bog" area, species richness did not vary among the different *Sphagnum* segments (on
210 average: 15 species), while in the "fen" area species richness significantly increased between
211 the upper segments (on average: 12 species) and the intermediate/lower segments (on
212 average: 15 species) ($P < 0.05$). In both areas, the highest diversities were measured in the
213 intermediate and lower segments (H'= 3.3), and the lowest diversity in the upper segments
214 ("fen": H'= 1.8; "bog": H'= 2.5). The equitability index also demonstrated a strong
215 dominance of some species in upper segments ("fen": J = 0.5; "bog": J = 0.7), while in the
216 intermediate and lower segments the communities were more balanced (both areas: J = 0.85).

217

218 Vertical micro-distribution

219 The NMDS ordination of samples from the two sampling areas showed that testate amoeba
220 communities differed significantly along *Sphagnum* segments in the two sampling areas (Fig.

1; $P < 0.001$). In the "fen" area the upper segment was clearly different from the intermediate and lower segments, while in the "bog" area this difference was less marked.

In the "fen" area, the most abundant taxa in the upper segments were *Archerella flavum* (on average 2.2 x 10^4 ind.g^{-1} DW) and *Hyalosphenia papilio* (on average 1.5 x 10^4 ind.g^{-1} DW) (Fig. 2 and supplementary Fig. 2). The intermediate segments were characterized by an increased of the abundance of *Hyalosphenia elegans* (on average of 8.3 x 10^4 ind.g^{-1} DW), *Nebela tincta* and *Physochila griseola* (both on average 3.5 x 10^4 ind.g^{-1} DW), and a significant decrease in the abundance of *A. flavum* and *H. papilio.* The lower segments were characterized by the highest abundance of *P. griseola* (on average 1.07 x 10^4 ind.g^{-1} DW) and *H. elegans* (on average 6.5 x 10^4 ind.g^{-1} DW).

In the "bog" area, the most abundant taxa in the upper segments were also *A. flavum* (on average 1.22 x 10^4 ind.g^{-1} DW), *N. tincta* (on average 3.8 x 10^4 ind.g^{-1} DW), *H. papilio* (on average 3.5 x 10^4 ind.g^{-1} DW), and *Assulina muscorum* (on average 8 x 10^4 ind.g^{-1} DW) (Fig. 2 and supplementary Fig. 2). The intermediate segments were characterized by significantly higher densities of *H. elegans* (on average 1.18 x 10^4 ind.g^{-1} DW), *N. tincta* (on average 1.0 x 10^4 ind.g^{-1} DW), *Amphitrema wrightianum* (on average 9.7 x 10^4 ind.g^{-1} DW) and *P. griseola* (on average 7.0 x 10^4 ind.g^{-1} DW) and lower density of *H. papilio*. In the lower segments, the most abundant taxa were *P. griseola* (on average 2.4 x 10^4 ind.g^{-1} DW) and *N. tincta* (on average 9.0 x 10^4 ind.g^{-1} DW).

Species-environment correlations

The multiple factor analysis (MFA) of the three environmental matrices and the three testate amoeba data sets confirmed the existence of an overall division between "fen" and "bog" areas (Fig. 3). The composition of testate amoebae community in the upper segments was

significantly linked to the chemical data and to testate amoeba assemblages of the intermediate segments (Table 3). The testate amoeba communities from the intermediate segments were significantly correlated to both chemical and phenolic data. No significant correlation was found between the testate amoeba communities of the lower segment and the environmental data sets. These patterns are further explored in the RDAs.

In the RDA ordinations (Fig. 4a, b, c and d), the two areas were clearly separated in the overall analysis as well as for each of the three sampling depths. The model explained 51.8% (adjusted r^2) of the variability in testate amoeba data in the overall analysis and 27.5%, 52.7% and 41.9% (adjusted r^2) of the variability in the data for the upper, intermediate and lower sections respectively. In the overall RDA, testate amoeba communities in the "fen" area were related to higher values of Eh, pH and W-temp, while testate amoeba communities in the "bog" area were related to higher values of phenolics, altitude and conductivity (Fig. 4a, b, c and d).

The RDA on individual environmental variables revealed that the proportion of testate amoebae data explained by each explanatory variable and the significance varied strongly among variables, between the two areas, and among the three vertical positions (Table 4). In the separate RDAs on the "fen" and "bog" samples all sampling depths were significant but no physical or chemical variable was found significant. Free phenolics explained a high proportion of variance in the upper and intermediate *Sphagnum* segments.

The partial RDAs showed that chemical, physical and phenolic data sets each significantly explained, independently of the other two data sets, about 7% of the species data variance (P = 0.02-0.08) in the overall RDA. The proportion of variance explained by these data was however much higher in the upper two segments (16.5–34.1%) but on average lower

in the third segment where no significant correlation was found for the lower segment (Table 5).

Discussion

Testate amoeba density and diversity

The communities of testate amoeba were dominated by representative of the *Amphitremidae* and *Hyalosphenidae*. This community composition is similar to the hummock fauna described by Heal [26, 27] along a fen-bog gradient. The similarities between these surveys are not surprising, and support previous studies in illustrating the cosmopolitan distribution of many peatland testate amoeba morphospecies from the same habitat type [43, 64]. Density is also similar to that reported in other studies on peatlands [20, 44].

Vertical micro-distribution

Testate amoebae reached their highest Shannon diversity and equitability in the intermediate and lower *Sphagnum* segments. The density of some taxa also differed significantly between the two sampling areas in some segments. The NMDS and RDA revealed contrasting vertical patterns of the testate amoeba communities especially in the fen area. *Archerella flavum*, *Heleopera sphagni* and *Hyalosphenia papilio* together represented between 57% (“bog”) and 88% (“fen”) of the total community in the upper segments, but much less in the intermediate and lower segments. Thus in agreement with previous studies [25, 34, 35, 39, 54], mixotrophic species largely dominated the community in the upper segments, while heterotrophic species (e.g. *P. griseola* or *Hyalosphenia elegans*) occurred principally in the intermediate and lower segments of *Sphagnum* in both areas.

The vertical micro-distribution of testate amoebae in *Sphagnum* reflects some gradients such as light, temperature, oxygen, prey organisms [35, 53]. A vertical niche separation among co-generic or otherwise closely related species also appeared in both sampling areas (e.g. the Amphitematidae *Archerella* and *Amphitrema*, and the Hyalospheniidae *Nebela, Hyalosphenia* and *Physochila*). This would support the idea of a competitive exclusion mechanism between closely related species of testate amoebae [44]. Mixotrophic species preferentially colonize the uppermost segments of *Sphagnum*, where their endosymbionts can photosynthesize [9, 25, 54]. Testate amoebae also need to find the required material to build their test, and this requirement may be another constraint that determines their vertical micro-distribution [35, 53]. For example, *Amphitrema wrightianum* and *Archerella flavum*, two closely related mixotrophic taxa, have an ecological niche separation along *Sphagnum* segments [25]. *A. flavum* produces a shell composed of self-secreted proteinaceous material whereas *A. wrightianum* uses xenosomes (e.g. organic debris, diatom frustules) [46]. This difference in shell construction explains the different vertical distribution pattern between *A. flavum* (upper segments) and *A. wrightianum* (intermediate segments) in the two sampling areas [43]. The source of material for test construction and the availability of appropriate food thus appear as major regulators of the abundance and the repartition of these species along *Sphagnum* parts [20, 25, 37]. In addition, these different constraints could also be taken into account to explain some species distribution patterns along micro-environmental gradients [43].

Species-environment correlations

Our results agree with earlier studies in identifying the fen/bog gradient as an important factor shaping the structure of testate amoeba communities [5, 27, 28, 34, 37, 38, 63]. Indeed in the "fen" habitat, *A. flavum*, *H. sphagni* and *H. papilio* were found in greatest abundance and

316 marked the ecological transition in *Sphagnum* upper segments. These species are typically
317 found in habitats with high (> 95%) soil water content [7, 30, 63]. Other species such as *N.*
318 *tincta* and *A. muscorum* described as xerophilous [12, 13] were more abundant in the "bog"
319 habitat. Nevertheless, DWT did not emerge as strongly correlated to testate amoeba
320 communities. The DWT gradient (ca. 3 cm) may not have been long enough to emerge as a
321 significant relationship. However other factors, including altitude, temperature, Eh,
322 conductivity, and free phenolics did explain a high proportion of the species data and all of
323 these were significantly different or nearly so between the two areas. Thus although DWT
324 almost always emerges as the strongest variable explaining testate amoeba community
325 structure in *Sphagnum* peatlands [3, 7], other variables become more important when the
326 DWT gradient is short.

327 Direct gradient analysis (RDA) with single explanatory variables revealed the
328 correlations of chemical factors (i.e. Eh and conductivity) with testate amoeba communities in
329 upper and intermediate segments. Water chemistry is known to influence testate amoebae
330 reproduction [25] and to contribute to changes in testate amoeba distribution [30, 42, 48], but
331 generally strongest correlations were reported with pH [41, 43]. Mieczan [39] demonstrated
332 that testate amoeba in the lower section (5-10 cm) were influenced by a combination of
333 chemical and physical factors (DWT and total organic carbon). Chemical factors explained a
334 high proportion of the testate amoeba data in the upper and intermediate segments, and their
335 influence decreased in lower segments. Testate amoebae from the upper segments were most
336 strongly correlated with the physical variables (i.e. altitude and water temperature) while in
337 the lowest segment, of all measured variables only water temperature and altitude were
338 significant. These results illustrate how vertical gradients lead to ecological niche separations
339 in *Sphagnum* peatlands.

340

341 Influence of phenolic compounds on testate amoeba communities

342 *Sphagnum* phenolics quantified in this work were extracted either water (free phenolics) or
343 solvent (bound phenolics) and the two methods yielded different results and patterns: bound
344 phenolics did not differ along the gradient whereas water-soluble phenolics did suggesting
345 that the amount of free phenolics may respond more strongly to micro-environmental
346 conditions (e.g. moisture content of mosses). These results also suggested that different kind
347 of phenolic compounds or phenolic concentrations characterized those extract. The
348 correlation between free phenolics and testate amoeba communities was particularly clear in
349 the upper and intermediate segments that correspond to the depth sampled for total
350 polyphenol analyses (0 – 6 cm). As the upper segment constitutes most of the biomass of
351 *Sphagnum* mosses owing to the weight of the capitulum (top 1 cm), most of the measured
352 phenols are contained in this segment. This may explain that the correlation between testate
353 amoebae and free phenols was highest in the upper segment and was also high in the
354 intermediate segment. We tentatively interpret the fact that no significant correlation was
355 observed between free or bound phenols and testate amoebae in the lower segment as an
356 indication that either the patterns of phenol concentration at that depth is not correlated with
357 that of the upper 6 cm or that the amoebae are more influenced by other aspects of water
358 chemistry closer to the water table. These results clearly call for a detailed analysis of
359 phenolics and testate amoebae at different depth, which could not be done at our site owing to
360 the limited amount of material that could be harvested in this long-term experiment.

361 Among competitive interactions, this study outlines potential chemical interaction
362 between *Sphagnum* and testate amoebae. Recently, phenolic compounds released by
363 *Sphagnum* mosses (e.g. *p*-hydroxyl phenolics) have been shown to possess antibacterial
364 activity [36]. Thus it is possible that free phenolic compounds play a role in testate amoeba
365 assemblages due to their selective positive or negative effects. Although results do not allow

366 to drawing any conclusions on a possible direct (e.g. physiological) and/or indirect (e.g.
367 through impact on prey organisms) effect of phenolics on testate amoeba communities, they
368 raise the issue of the possible role of such compounds. An experimental approach to test such
369 effects is necessary.

370

371 Conclusions

372 In this study we explored the community patterns and species-environment relationships of
373 testate amoebae living in *Sphagnum fallax* along a narrow ecological gradient from a poor fen
374 (homogeneous *Sphagnum* carpet) to a "young bog" (mosaic of poor fen and bog microsites).
375 In agreement with our hypotheses we observed differences between the two sampled habitats
376 and a vertical stratification of communities. These results illustrate how strongly microbial
377 communities respond even to short ecological gradients in *Sphagnum*-dominated peatlands.
378 The analysis of testate amoebae from three *Sphagnum* segments allowed us to explore the
379 detailed patterns of species-environment relationships at the time of sampling and showed
380 that slight environmental variations (e.g. altitude and related variables) are significant at the
381 microbial level. This study therefore confirmed that testate amoebae are sensitive to
382 environmental gradients at a very fine scale [40]. The importance of temporal patterns also
383 would deserve more attention. Indeed, the location and size of different microhabitats and
384 related communities in *Sphagnum* peatlands are not stable over time [8] and this is clearly
385 also true for testate amoeba assemblages as attested by the limited existing data on seasonal
386 patterns [62] as well as the changes documented in numerous palaeoecological records [10].
387 Understanding environmental controls on testate amoebae communities at these finer spatial
388 and temporal scales is key to improving our ability to interpret the high-resolution fossil
389 testate amoeba records in peatlands that is starting to being produced [31]. This will require

both further detailed descriptive studies as well as manipulative experiments using biotic (phenols) and abiotic data and aiming to determine which factors influence testate amoebae and what the mechanisms are.

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Tables

Table 1. Environmental variables measured in the “fen” and “bog” sampling areas in Le Forbonnet mire (French Jura) (n = 12, average ± S.E).

Table 2. Non-parametric correlation matrix of measured environmental variables along the “fen”/”bog” transition of Le Forbonnet mire.

Table 3. RV-coefficients (below diagonal) and corresponding *P*-values (above diagonal) among the six groups of variables used in the MFA of the Forbonnet peatland. Significant coefficients appear in bold.

Table 4. Summary of RDA on testate amoebae and environmental variables from Le Forbonnet mire (France): fraction of variance explained and significance of individual variables taken alone.

Table 5. Summary RDA and variance partitioning on testate amoebae and environmental variables data from Le Forbonnet mire (France).

Figures

Figure 1. (a) The two primary axes of the 3-dimensional NMDS ordination of testate amoebae communities in the "bog" area from Le Forbonnet mire (France) (n = 18, final stress = 4.1). The solution represents 75% of the variability in the data, with axes 1, 2 and 3 representing respectively 43%, 18% and 13%. Samples are coded by sampling area with open symbols. (b) The two primary axes of the 3-dimensional NMDS ordination of testate amoebae communities in the "fen" area (n = 18, final stress = 2.4). The solution represents 84% of the variability in the data, with axes 1, 2 and 3 representing respectively 55%, 19% and 10%. Samples are coded by sampling area with filled symbols.

Figure 2. Distribution maps of total testate amoeba abundance and of dominant testate amoeba taxa in *Sphagnum fallax* from the two sampling areas in Le Forbonnet mire (France). A = upper (0-3cm) B = intermediate (3-6cm) and C = lower (6-9cm) segments. X and Y axes correspond to GPS data converted into Lambert 2 references. Dot sizes are directly proportional to the number of individuals per gram DW in the samples and are comparable among maps.

Figure 3. Multiple factor analysis of the three testate amoeba communities (Hellinger-transformed) and environmental (chemical, physical and phenolics) data sets from the Forbonnet peatland. Projection of the MFA axes 1 and 2 with the result of a hierarchical agglomerative clustering (grey lines), obtained by the Ward method on the Euclidean distance matrix between MFA site scores, showing two main groups of sampling plots (open symbols = "fen", filled symbols = "bog"). Sampling plots are indicated by F ("fen") or B ("bog") followed by a number.

Figure 4. Redundancy analyses biplots (axes 1 and 2) of testate amoeba data from Le Forbonnet mire (France) in upper (a), intermediate (b) and lower (c) *Sphagnum* segments, and the overall data set (d). Sampling areas are coded with open symbol for the "fen" area and with filled symbol for the "bog" area. Samples are indicated as follows: circles = upper segments, squares = intermediate segments, triangles = lower segments. F_phe : free phenolics; B_phe : bound phenolics; W-temp: water temperature; Alt: average altitude (microtopography) of the sampled plot; Kcorr: conductivity.

Supplementary Online Material

Supplementary Figure 1. Location of the Forbonnet peatland with inset showing the location of the sampling areas.

Supplementary Figure 2. Vertical micro-distribution of selected testate amoeba taxa in the two sampling areas (average ± S.E) (circles: “bog” area; triangles: “fen” area). Asterisks indicate significant differences between the sampling areas ($P < 0.05$). Different letters indicate significant differences among *Sphagnum* sections ($P < 0.05$).